User manual

Check-Direct CPE for BD MAX™

Real time PCR kit for the detection of carbapenemase-producing *Enterobacteriaceae*

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Intended use

Check-Direct CPE is a qualitative *in vitro* diagnostic test for the rapid detection of carbapenemase genes KPC, VIM, OXA-48 and NDM, presently the primary cause of carbapenemase production in *Enterobacteriaceae*. The assay is performed on the BD MAX™ system using bacterial cell suspension from clinical specimen of individuals at risk of colonization with carbapenemase-producing *Enterobacteriaceae*. Check-Direct CPE is not intended to diagnose infections with carbapenemase-producing *Enterobacteriaceae* nor to guide or monitor treatment for these infections. Parallel cultures are necessary to recover organisms for epidemiological typing, susceptibility testing and for further confirmatory identification.

Introduction and principle of the method

The worldwide emergence and dissemination of carbapenem resistance among *Enterobacteriaceae* is a serious threat to public health. These organisms are associated with high mortality rates and have the potential to spread widely. The most common cause of carbapenem resistance in *Enterobacteriaceae* is the expression of carbapenemases, *i.e.* Carbapenemase-Producing *Enterobacteriaceae* or CPE. CPE have elevated or complete resistance to carbapenems and most other β-lactam antibiotics. Presently, the vast majority of CPE are associated with the presence of one of the following plasmid-encoded carbapenemases: KPC (*Klebsiella pneumoniae* carbapenemase), VIM (Verona integron–encoded metallo-β-lactamase), NDM (New Delhi metallo-β-lactamase) or OXA-48 (Oxacillinase-48). Moreover, CPE often have other non–β-lactam resistance determinants resulting in multidrug- and pandrug-resistant isolates.

Check-Direct CPE is a multiplex real-time PCR assay for detection of the KPC, OXA-48, NDM and VIM carbapenemase genes. The assay is based on specific recognition and amplification of target sequences by PCR, and the simultaneous detection of the accumulation of PCR amplification products by fluorescent DNA probes. Four molecular beacon probes labelled with four different dyes are used for the fluorescent detection and the discrimination of the carbapenemase markers. For each of the 4 carbapenemase genes, KPC, VIM, OXA-48 and NDM, many gene variants exist. PCR primers and fluorescent probes of Check-Direct CPE are selected to target homologous gene segments of these carbapenemase genes, and in this way all gene variants are reliably detected.

Kit contents (for 48 reactions)

Components (Mat. No.)	Description	Storage conditions
CPE solution (9-0072)*	1 brown tube (blue inlay ●) 550 µl	- 20°C, **store in the dark**
Negative control (9-0070)	1 tube (white inlay ○) 100 µl	
KPC positive control (9-0073)	1 tube (green inlay ●) 100 µl	
VIM positive control (9-0074)	1 tube (yellow inlay ●) 100 µl	
OXA-48 positive control (9-0076)	1 tube (orange inlay ●) 100 µl	
NDM positive control (9-0075)	1 tube (gold inlay ●) 100 µl	
User Manual (9-0079)	Leaflet – download from website	Not critical

Storage, handling, and stability

The Check-Direct CPE kit is shipped cooled and should be stored immediately at -20°C upon receipt. Store all opened reagents at -20°C until expiration date. Store in the dark. Please visually inspect the box upon initial opening to ensure that its contents are intact.
The CPE solution should not be exposed to more than 12 freeze-thaw cycles.
Please contact the Check-Points office at *support@check-points.com* if you have any further questions.

Materials required but not supplied with the kit

Supplies	Equipment
• BD MAX™ ExK™ DNA-1 Extraction Kit (Ref:442818) • BD MAX™ DNA MMK (SPC) Master Mix (Ref: 442829) • BD MAX™ PCR Cartridges (Ref: 437519) • Disposable laboratory (powder-free) gloves • Lab coat • Pipettes & disposable (filter-) tips for volumes of 1 to 1000 µl • DNAase free 1.5 ml tubes (1.5 and/or 2ml) • PCR-grade water (e.g. Milli-Q)	• Real-time PCR instrument: BD MAX™ System, software version 2.92 • Vortex mixer • Mini centrifuge

Good laboratory practices

Recommendations for best results

The quality of the results depends on strict compliance with the following good laboratory practices, especially concerning PCR practices.

- The test must be performed by adequately trained personnel.
- Do not use reagents after their expiration date
- Before use, thaw frozen reagents completely at room temperature and vortex briefly to obtain a homogeneous solution. After vortexing briefly, spin down the solution to avoid contamination when opening the lid. Avoid unnecessary freeze-thawing of the kit content.
- Follow recommendations for storage, handling and freeze-thaw cycles to preserve the quality of the kit's reagents.
- Protect reagents from light to avoid photo-bleaching of the dyes.
- Periodically, verify the accuracy and precision of pipettes, as well as correct functioning and calibration of the instruments.

Prevention of contaminations

Use separate rooms: a pre-PCR room and a post-PCR room.

- Preparation of the amplification reactions are carried out in the pre-PCR room.
- DNA extraction and real-time PCR assays are carried out in the post-PCR room.
- Never transfer items from the post-PCR room to the pre-PCR room.

To keep laboratory free of PCR product contamination:

- Use pipettes with hydrophobic filter tips.
- Make sure to always use a new pipette tip when adding solutions, test samples, and controls to a reaction tube to avoid contamination.
- Follow proper pipette-dispensing techniques to prevent aerosols.
- Wear clean disposable gloves and clean lab coats for the different steps of the test.
- Change gloves whenever you suspect that they are contaminated.
- Keep the tubes of all kit components and samples closed as much as possible.
- Clean the lab benches and all equipment regularly with a 0,5% sodium hypochlorite solution.

Users are strongly advised to read the full protocol before starting the test

Specimen and cell suspension preparation

- Inoculate nutrient agar plates with the clinical samples or the bacterial strains to be tested and incubate overnight at 37°C. Typical growth media include blood agar, MacConkey agar and Tryptic Soy agar.
- Prepare a bacterial cell suspension of McFarland 0.5 (≈1 x 10^8 CFU/ml) in cell suspension buffer (*e.g.* PBS or 10mM Tris HCl pH8.0) or PCR-grade water from bacterial cells grown on the agar plate. Dilute the suspension 100 times to obtain a suspension of ≈1 x 10^6 CFU/ml.

BD MAX™ sample and control preparation

Important points before starting: Refer to the BD MAX™ ExK™ DNA-1 kit user manual and BD MAX™ System User's Manual for detailed instructions.

1. Sample preparation

- After specimen collection, transfer the bacterial cell suspensions to be analyzed in the sample preparation area.
- Pipette 500 µL of the bacterial cell suspension (≈1 x 10^6 CFU/ml) into one DNA Sample Buffer Tube SB-1 (supplied by BD with the DNA extraction kit, refer to *Material required but not supplied with the kit*).
- Close the Sample Buffer Tube with a septum cap and vortex 10 second at low speed.

2. Positive and negative control preparation

To validate the run, perform positive and negative control reactions for each Check-Direct CPE PCR run. The positive and negative controls are supplied with the kit.

- **Positive control(s)**
  Test the relevant positive control(s) provided in the kit. Pipette 10 µL of positive control (KPC (●), VIM (●), OXA (●) or NDM (●)) and 500 µL of PCR-grade water into one Sample Buffer Tube. Vortex for 10 seconds. Positive controls may also be combined: add 10 µL of each positive control to be tested and 500 µL of PCR-grade water into one Sample Buffer Tube. Vortex for 10 seconds.
- **Negative control(s)**
  Pipette 10 µL of the negative control (solution O) provided in the kit and 500 µL of PCR-grade water into one Sample Preparation Reagents tube. Vortex for 10 seconds.

BD MAX™ system real-time PCR

1. Multiplex real-time PCR (qPCR) setup

Table 1 presents the multiplex qPCR setup with the targets detected in each detector channel of the BD MAX™ System.

Table 1: *Multiplex qPCR setup*

Detector	475/520	530/565	585/630	630/665	680/715
Channel	1	2	3	4	5
Target	KPC	VIM	OXA-48 like	NDM	SPC*

*SPC: Sample Preparation Control

2. qPCR Mix preparation

2.1 Calculate the number of reactions for the specimen and controls (maximum 24 samples in total in one run). Thaw reagents, mix well, spin down and keep on ice.
2.2 Prepare the qPCR reaction mix as described in Table 2 using the CPE solution provided in the Check-Direct CPE kit and the BD diluent provided in the BD MAX™ DNA MMK (SPC) reaction kit. Multiply the CPE solution and the BD diluents solutions by the total number of reactions calculated, plus 10% surplus to ensure that you have enough qPCR reaction mix for all reactions.

Table 2: *qPCR reaction mix*

Component	Volume per reaction strip
BD Primer and Probe Diluent (12.5X)	2 µl
CPE solution (●)	10,5 µl
Total volume for 1 strip	12,5 µl

3. Creating the Check-Direct CPE test

Important points before starting: Refer to BD MAX™ System User's Manual for detailed instructions on how to operate the BD MAX™ System and software v2.92. Skip step 3. if a PCR test program has already been created for the Check-Direct CPE test.

3.1 Create a new Test, select **Create test**, and enter the following parameters:

- Test Name: type *Check-Direct CPE*
- Extraction Type: select *Exk DNA-1*

- Master Mix Format: choose *Type 2 MMK (SPC), Probes/Primers Add Manually*
- Channel detector Settings: set *Gain* and *Threshold* with parameters presented in Table 3
- GardRail: select Default
- Test details: enter the PCR profile, see Table 4
- Spectral Cross Talk tab: enter parameters presented in Table 5

Table 3: *Gain parameters.*

Detector	Gain	Threshold
475/520	40	100
530/565	80	150
585/630	30	200
630/665	80	300
680/715	40	300

Table 4: *Real-time protocol parameters.*

Step Name	Profile Type	Cycles	Time (s)	Temp(°C)	Detect
Denaturation	Hold	1	600	98	NO
Step Name	Profile Type	Cycles	Time (s)	Temp(°C)	Detect
Amplification &Detection	2 - temperature	45	15	98	NO
			62	60	YES

Table 5: *Spectral cross-talk parameters.*

		False Receiving Channel				
		475/520	530/565	585/630	630/665	680/715
Excitation Channel	475/520		0.0	0.0	0.0	0.0
	530/565			0.0	0.0	0.0
	585/630	0.0			7.4	0.0
	630/665	0.0	0.0	0.0		0.0
	680/715	0.0	0.0	0.0	4.4	

3.2 Select **Save Test.**

4. BD MAX™ Rack set-up

4.1. Load the BD MAX™ system racks with the number of DNA Unitized Reagents Strips necessary for the number of samples to test. Gently tap each strip to make sure all liquids are at the bottom of their container.

4.2. Prepare Unitized Reagents Strips:

4.2.a Snap the appropriate DNA extraction Reagent tube (white cap) into positions 1 into the DNA Strip, see Figure 1.

4.2.b Snap in a DNA MMK (SPC) Master Mix tube (green/yellow cap) into position 2 of the DNA strip, see Figure 1.

4.2.c Snap a Conical Tube into position 3 (Figure 1). Pipette 12.5 µl of qPCR mix (Table 2) into the Conical Tube in position 3. Avoid bubbles and make sure the liquid is at the bottom of the tube.

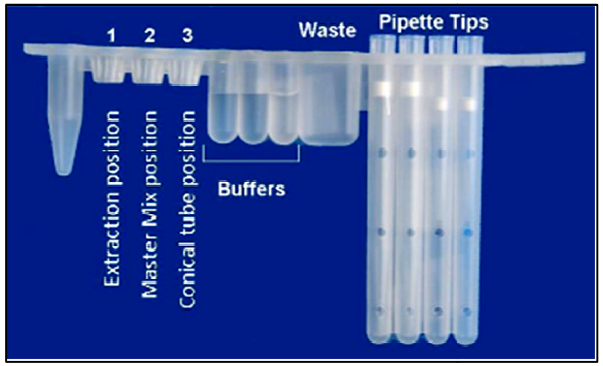

Figure 1: *DNA Unitized Reagent Strip setup.*

5. BD MAX™ instrument set-up

5.1 Open the **Run** screen of the BD MAX™ System software v2.92.
5.2 In the **Assay** menu select *Check-Direct CPE*.
5.3 Enter the Sample Preparation reagent tube barcode using the barcode scanner (you can also enter the barcode manually). Start with position 1 of rack A.
5.4 Place each of the Sample Buffer Tubes in their corresponding position in the BD MAX™ racks (with septum cap).
5.5 Enter the specimen or patient identification information into the work list. Check that each specimen or patient information correspond to its specific Sample Buffer Tubes in the Rack.
5.6 Load the Rack(s) into the BD MAX™ System. (Rack A is positioned on the left side of the instrument and Rack B on the right side).
5.7 Load the BD MAX™ PCR cartridge(s).
5.8 Close the instrument door and select **Start Run.**

BD MAX™ Results

Important points before starting: For a detailed description on how to analyze data, refer to *BD MAX™ System User's manual*.
Always visually inspect the amplification plot for each sample tested versus C_T values obtained with the software.

1. How to create and print data report

1.1 In the main menu, select **Results** and double click on the run to be analyzed.
1.2 Select the Run to analyze.
1.3 In the **Print tab** select the box for **Results Protocol Details and PCR.**
1.4 Select **create report**. The report can be saved as pdf file format. To print it select **Print Report.**

2. Analysis

The BD MAX™ software reports C_T values and amplification curves for each detector channel of each specimen tested in the following way:

- C_T value of **0** indicates that there was no C_T value calculated by the software. Amplification curve of the sample showing a "0" C_T value must be checked manually.
- C_T value of **-1** indicates that no amplification process has occurred. Check that there is no amplification curve for the sample with a C_T value of -1 on the graphical results.
- Any other C_T value should be interpreted in correlation with the amplification curve (**PCR Analysis** tab) and according to the interpretation method, see Table 6-7

3. Interpretation

3.1 Run validation

Verify that the real-time PCR run is valid before data interpretation of the results. Check that there is no report of BD MAX™ System failure. Check the positive and negative control amplification curves. Table 6 shows criteria for a valid real-time Check-Direct CPE run on the BD MAX™ System.
Expected C_T values for the positive and the negative controls are given as an indication not a definitive result. These values will depend on the threshold set by the software. If the controls C_T values are not as expected refer to FAQ and Troubleshooting **"3"**.

Valid run reports:

- No BD MAX™ System failures during the run
- Positive controls with a C_T value for the carbapenemase targets and sample processing control (SPC) as depicted in Table 6.
- Negative control with a C_T value of -1 in channel 1 (475/520), 2 (530/565), 3 (585/630) and 4 (630/665) and a C_T value of 29 ±3 for the sample processing control (SPC) in channel 5 (680/715).

Table 6: *Criteria for a valid run with Check-Direct CPE test.*

Sample Type*	C_T 475/520 KPC	C_T 530/565 VIM	C_T 585/630 OXA-48 like	C_T 630/665 NDM	C_T 680/715 SPC
Positive controls	32 ±3	30 ±3	29 ±3	31 ±3	29 ±3
Negative sample	-1	-1	-1	-1	29 ±3

* If observed CT values vary significantly from expected CT values , see FAQ and Troubleshooting section

3.2 Results interpretation

If the run has been validated, interpret results as positive, negative or invalid with the C_T values obtained for the samples following the guidelines outlined below and summarized in Table 7.

- Positive carbapenemase samples will show a C_T value in channel 1 (475/520), 2 (530/565), 3 (585/630) and/or 4 (630/665). Positive carbapenemase samples will also show a C_T value for the SPC in channel 5 (680/715).
- Negative carbapenemase samples will show no C_T value in channel 1 (475/520), 2 (530/565), 3 (585/630) and 4 (630/665). In channel 5 (680/715) a C_T value for the SPC is expected of 29 ±3.
- Samples with carbapenemase target C_T of -1, and with a SPC showing no C_T value or a C_T ≥ 32 means that the assay is not valid and should be repeated, see FAQ and Troubleshooting 3 to 6.

Table 7: *Data interpretation guidelines.*

KPC, VIM, OXA, NDM C_T values	SPC C_T values	Interpretation
YES	YES	Positive sample
-1	29 ±3	Negative sample
-1	-1 or >32	Invalid

Frequently asked questions (FAQ) & Troubleshooting

Refer to “the troubleshooting” section of the BD MAX™ System User’s Manual for additional information

1. **Real-time results show no C_T values or interpretation indicating that the sample is invalid.** Possible causes and troubleshooting:
   - The PCR reaction has been inhibited by exogenous or endogenous substances. Please repeat sample testing.
   - The sample tested with Check-Direct CPE is negative and the internal control was not amplified.
   - The DNA extraction failed since the SPC was not detected.
   - CPE Solution or The BD MAX™ DNA MMK (SPC) PCR mix was not added to the assay. Please repeat the test.
   - The BD MAX™ DNA MMK (SPC) PCR mix may have expired.
   - A error in liquid handling has occurred: check unitized reagent strips and microfluidic cartridge to determine where liquid handling problem has occurred (example: air bubble in the cartridge) and re-run the sample. If the problem persists, contact your local BD representative.

2. **Troubleshooting for invalid results.**
   For Invalid results: Repeat test with the original specimen by preparing a new Sample Buffer Tube. Alternatively, test newly collected specimen.

3. **Real-time results show no C_T values for the positive control or interpretation indicating that sample is invalid?**
   Possible causes and troubleshooting:
   - The positive control solution was not added.
   - qPCR reaction mix (CPE Solution with Primer/Probe Diluent) was not added to the assay. Please repeat the test.
   - The BD MAX™ DNA MMK (SPC) PCR mix was not added or may have expired.

4. **Real-time results show very low fluorescent signals in all samples and detector channels, including the internal control signal.**
   Possible causes and troubleshooting:
   - The CPE solution containing the fluorescent probes and primers is degraded. Please check expiration date, the number of thaw/freezing cycle that the CPE solution tube has undergo, and if the kit has been stored correctly.
   - The BD MAX™ System can be responsible for these results. Please refer to BD MAX™ User’s manual or contact your BD local representative.

5. **The BD MAX™ System states an error or failure.**
   Refer to the BD MAX™ instrument user manual or contact your BD local representative.

6. **All amplification curves show a spike at the same point in time.**
   A problem with the power supply may have occurred. Verify that the system is correctly connected to its un-interruptible power supply and repeat experiment.

7. **I have left Solutions (CPE or Positive control) out of the -20°C (-4˚F) storage.**
   These reagents must be stored at -20°C (-4°F) for proper performance of the test. The performance of the product cannot be fully guaranteed if these solutions were left out of -20°C (-4˚F) for more than 24 hours.

8. **Duplicate samples tested with Check-Direct CPE test do not yield identical results.**
   C_T values of identical samples may vary between individual reactions. Large variations, > 2 C_T values, suggest pipetting errors or other differences between the duplicate samples.

Limitations

Check-Direct CPE uses a range of specific DNA markers to detect the presence of the carbapenemase genes KPC, NDM, OXA-48, and VIM, which currently represent the clinically most prevalent carbapenemases. The test detects all presently known variants of KPC, NDM, OXA-48 and VIM, except VIM-7, a rare variant only found in *Pseudomonas aeruginosa*. It should be noted that other rare carbapenemase gene families are not detected.

Check-Direct CPE was developed for bacterial cell suspension. The quality of the input DNA is an important factor for obtaining reliable results with Check-Direct CPE. DNA must be extracted from bacterial cell suspension validated with Check-Direct CPE and described in this manual. The assay has been tested extensively with purified DNA from gram-negative bacteria, such as *Escherichia*, *Salmonella*, *Klebsiella*, *Enterobacter*, *Citrobacter* and *Pseudomonas*, with excellent results. However, it may never be excluded that other Gram-negative bacteria or certain strains of the above species will yield poor results. Check-Direct CPE cannot and does not make any representation or warranty that it is capable of correctly detecting the carbapenemase genes in all gram-negative species, subspecies or types or in all clinical sample source. Results may need to be confirmed by additional methodologies in specific cases (e.g. for regulatory samples). Due to the high variability of bacterial genomes it is possible that certain subtypes might not be detected. The test reflects the state of knowledge of Check-Points Health B.V.
The presence of multiple bacterial species in a sample may hamper the interpretation of the test. As with other diagnostic assays, the results of this test may only be interpreted in combination with additional laboratory and clinical data available to the responsible person. Use of this assay is limited to appropriately qualified personnel, well-trained in performing DNA-based molecular detection methods.

Technical assistance

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Key to symbols used

IVD	For *In Vitro* Diagnostic Use	CONTROL –	Negative control
REF	Catalog number	CONTROL KPC	KPC positive control
LOT	Batch code	CONTROL VIM	VIM positive control
IFU	IFU number	CONTROL NDM	NDM positive control
SOLUTION CPE	CPE solution	CONTROL OXA-48	OXA-48 positive control
	Consult instructions for use		Use before YYYY-MM
	Temperature limitation		Manufacturer
Σ	Contains sufficient for < n > tests		

Despite the utmost care in the development and preparation of the protocol Check-Points cannot take any responsibility for errors, omissions and/or future changes herein.

Literature Citation: When describing a procedure for publication using this product, please refer to it as the *Check-Direct CPE.*
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